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ANIMAL MODEL OF HUMAN DISEASE

The Non-Obese Diabetic (NOD) Mouse

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Biologic Features

The Non-Obese Diabetic (NOD) is an inbred mouse strain developed in the course of a breeding program to establish a cataract-prone subline (CTS) from non-inbred ICR mice.[1] The NOD subline is not cataract-prone; the first case of insulin-dependent diabetes (Idd) was observed in a NOD female at the 20th generation of inbreeding. After a further 6 generations of inbreeding, a cumulative diabetes incidence by 30 weeks of age of 60–80% in females versus only about 10% in males was observed.[1] Clinical features of the diabetes syndrome in NOD mice are quite similar to human Type 1 insulin-dependent diabetes mellitus (IDDM) and include abrupt onset between 90 and 120 days (equivalent to early adolescence period in humans), hyperglycemia, glycosuria, hypercholesterolemia, ketonuria, polydipsia, polyuria, and polyphagia.[1] Prospective analysis of changes in nonfasting blood or plasma glucose concentrations of NOD mice in our colony shows a gradual, rather than an abrupt, shift, with blood glucose rising from a normal level of approximately 140–160 mg/dl to permanent hyperglycemia (>400 mg/dl) over a period of 3–4 weeks. Glycosuria (read by Tes-Tape, Eli Lilly) usually denotes a plasma glucose of 300 mg/dl. Some NOD mice in our colony diagnosed as diabetic on the basis of a continuously rising nonfasting blood glucose level have exhibited transient remission from hyperglycemia (1–2 weeks in duration), a phenomenon suggestive of the "honeymoon" period noted shortly after diagnosis of some human Idd cases. Stepwise loss of beta cell capacity to respond to glucose and arginine correlates with declining pancreatic insulin content and increasing severity of histopathologic lesions in the pancreas.[2] A remarkable feature of this model is that onset of hyperglycemia can apparently be prevented by daily injections of prediabetic mice with nicotinamide, a compound known to protect mice from streptozotocin-induced diabetes.[3] NOD mice resemble mice made diabetic by multiple low doses of the diabetogenic antibiotic streptozotocin in terms of their ability to survive without insulin treatment for 1–2 months after detection of glycosuria, possibly reflecting secretion from residual beta cells in the pancreas or production by extrapancreatic tissues of molecules with insulinlike reactivity. Tissues from NOD mice retain sensitivity to exogenous insulin,[4] and insulin administration to diabetic NOD mice prevents weight loss.[1]

NOD females become permanently hyperglycemic at an earlier age than males (peak onset between 16 and 20 weeks as compared with 21 and 28 weeks) and at higher frequency. Gonadal sex steroids are important modulators of pathogenesis: orchiectomy increases, and ovariectomy reduces, diabetes incidence.[5] Environmental components, including diet, also modulate disease development: diabetes incidence, particularly in males, appears to vary considerably among different colonies of NOD mice. For example, the high cumulative diabetes incidence (by 40 weeks of age) of between 50% and 70% observed in

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NOD/Lt males in our colony can be reduced to zero by a chemically-defined (high fat) diet in place of our standard semi-defined diet (formulation 96W, Emory Morse Co., Guilford, Conn).

Insulitis, the infiltration of leukocytes into pancreatic islets, is the most prominent histopathologic lesion in NOD mice.[1] These infiltrates are found in both sexes shortly after weaning,[5] and contain both T and B lymphocytes.[6] The infiltrating cells usually originate from efferent capillaries and from lymphatics at the periphery of the islets and initially constitute a multilayered aggregation around the perimeter of the islets (Figure 1). In pancreases of chronically diabetic mice, the infiltrates have generally regressed; immunocytochemical staining shows islet residua to contain non-beta endocrine cell types. The inflammatory lesion is primarily associated with loss of beta cells, because levels of pancreatic glucagon and somatostatin increase concomitant to decreases in numbers of beta cells and pancreatic insulin levels.[7,8] Lymphoid cell aggregations are also found in the submandibular portion of the salivary gland in both sexes, as well as in the lacrimal and Harderian glands.[9] Insulitis is not predictive of eventual development of overt diabetes, because 100% of NOD males exhibited insulitis by >30 weeks of age in a colony in which incidence of overt diabetes was only 10%.[5]

A polygenic basis for diabetes susceptibility in the NOD/Lt strain has been established by outcrossing to a diabetes-resistant related inbred strain, Non-Obese Normal (NON), separated from the NOD line at the 6th generation of inbreeding.[1] A minimum of three recessive genes contribute to diabetes development in NOD mice.[10,11] The first one discovered,[12] and provisionally designated *Idd-1,* is tightly linked to the *K* locus of the *H-2* complex on Chromosome 17, but is not necessarily within the major histocompatibility (MHC) complex.[11] The second, *Idd-2,* is linked to the *Thy-1* and *Alp-1* loci on Chromosome 9.[10,11] The third locus has not yet been mapped, but can be shown to segregate in a second backcross to NOD by the use of mice of the first backcross typed for homozygosity for the NOD marker alleles. In addition to these recessive alleles, a gene or genes from NOD stimulating T lymphocyte proliferation is inherited by F1 and backcross mice in a dominant fashion; possibly, this could be a function of the unique NOD *I-A* region gene.[10-12]

Insulitis, coupled with the requirement for a diabetogenic recessive gene tightly linked to the MHC complex, suggested an autoimmune etiopathogen-

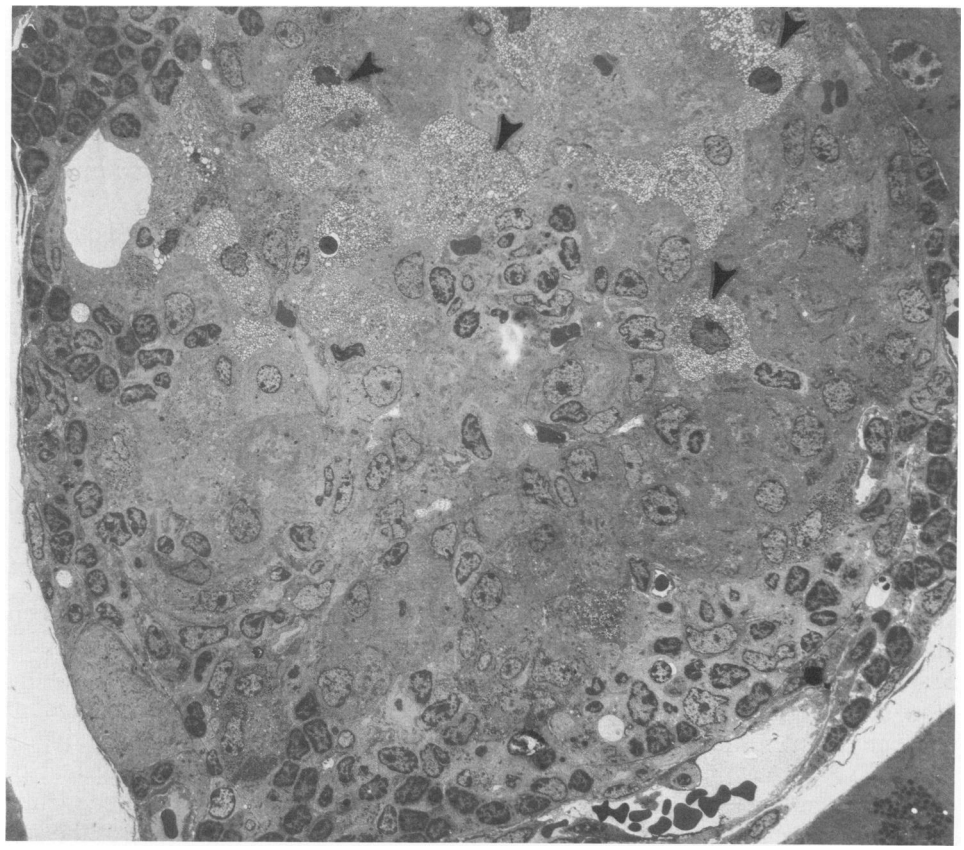

Figure 1—Electron micrograph of an islet from a 14-week-old aglycosuric NOD female showing perinsular aggregations of small lymphocytes. Cytopathic dilatation of rough endoplasmic reticulum is apparent in some of the beta cells (*arrowheads*). (×862)

esis. Moreover, the greater female susceptibility to diabetes induction indicates autoimmune etiology because lupus-prone female mice of strain NZB generally exhibit higher autoantibody levels and, consequently, earlier onset of disease than observed in males. Both cellular and humoral autoimmune reactivity against islet cells has been documented in NOD mice.[13] T lymphocytes are required for diabetes pathogenesis in this model inasmuch as NOD-*nu/nu* mice do not develop diabetes unless reconstituted with splenocytes from euthymic littermates and concomitantly treated with interleukin-2 (IL-2).[14] Moreover, diabetes was accelerated by transferring splenocytes from older NOD mice into irradiated 7-week-old recipients,[15] or by cyclophosphamide treatment.[3] Although one study reported NOD mice to be T-lymphopenic,[16] this finding has not been confirmed in other laboratories. On the contrary, a markedly increased concentration of T lymphocytes in spleen and peripheral blood is characteristic of NOD, but not NON mice.[11,17] Enlargement of the thymic cortex and lymph nodes reflect this lymphoproliferative stimulus associated with underlying immunoregulatory defects. Although NOD mice exhibit strong T and B lymphocyte responses to mitogens, there is a defect in their ability to generate suppressor T lymphocytes in a syngeneic mixed lymphocyte reaction.[17] Both monocyte and NK functions have been reported to be impaired in NOD mice,[16] and we have found endotoxin-stimulated IL-1 production from NOD macrophages to be low. Defective communication between macrophages and T-helper cells may account for the defective suppression mechanisms and thus the apparent hyperplasia of T lymphocytes in lymphoid tissues. This defect in generation of suppressor cells may be associated with a decreased endogenous production of IL-2, because either concanavalin A-stimulated splenocytes or a syngeneic mixed lymphocyte reaction supplemented with IL-2 allows generation of T suppressor-inducer cells, thus reversing the defect.[18]

Comparison With Human Disease

Idiopathic Type 1 IDDM in humans is thought to entail an autoimmune pathogenesis, with insulitis and association with certain marker haplotypes at the MHC complex (HLA) being pathognomonic. Makino has emphasized the similarities between the pathophysiology and histopathology of diabetes in the NOD mouse with IDDM[1] and has noted that the polyglandular distribution of leukocytic infiltrates in submandibular, lacrimal, and Harderian glands is suggestive of lesions observed in Sjögren's syndrome in man.[9] The polygenic control of diabetogenesis in NOD mice, coupled with the requirement for a diabetogenic susceptibility gene linked to the MHC complex, suggests close similarily between the genetics of this form of diabetes in NOD mice and Type 1 IDDM in man.

Usefulness of the Model

The diabetic NOD mouse provides a useful complement to the other well-studied rodent model of Idd, the spontaneously diabetic BB rat, in defining a diabetes-susceptible genotype.[19] Both rodent models share a requirement for an MHC-linked recessive diabetogenic locus, but the T-lymphopenia characteristic of most BB rats is not a trait shared by NOD mice, or, for that matter, most diabetic persons. Nevertheless, both NOD mice and BB rats may share a similar defect in T-lymphocyte suppression mechanisms which permit polyclonal B-lymphocyte activation and autoimmunity against pancreatic beta cells. In that the prodromal phase of diabetogenesis in NOD mice resembles that of prediabetic persons in terms of declining first-phase insulin secretion in response to glucose,[2] coupled with the development of insulin autoantibodies and autoantibodies against endogenous beta-cell retrovirus,[20] NOD mice are valuable not only for elucidating the pathogenesis of Type 1 IDDM in man, but in designing and testing effective therapies for prevention of the disease.

The mouse genome is the most extensively characterized of all mammalian species, and the availability of genetic markers in NOD mice has permitted identification of a second, non-MHC-linked locus required for diabetogenesis. Because there is considerable homology between linkage groups in mouse and man, identification of loci required for diabetogenesis in mice may indicate where homologous genes reside in the human genome. For example, the human homologs for *Thy-1* and *Alp-1*, two marker genes linked to *Idd-2* on mouse Chromosome 9, are on the long arm of Chromosome 11, while the human homolog of *Mod-1*, a distal marker gene for *Idd-2* on mouse Chromosome 9, is on human Chromosome 6 (but not on the same arm as the HLA locus).

Although the NOD mouse model is of obvious value in defining the polygenic nature of IDDM inheritance in man, and in elucidation of pathogenetic mechanisms, there are intrinsic differences in the biology of the respective species such that the pathophysiologic changes associated with diabetes in the mouse may not be identical to those in humans. For example, some of the secondary pathologic changes

associated with the morbidity of chronic IDDM in humans, such as kidney lesions, neuropathies, and retinopathy, have not yet been reported in chronically diabetic NOD mice.

Availability

NOD breeding pairs may be obtained from CLEA JAPAN, Inc., Aobadai, Meguro-ku, Tokyo 153, Japan (contact Mr. Y. Taguchi, Manager, International Division). NOD breeders produce large litters; care should be taken to maintain brother × sister matings to preserve the inbred status of the strain.

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